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HABITAT, SEED DORMANCY, AND ALLOZYME VARIATION OF THE RARE ENDEMIC *PHACELIA COOKEI* (BORAGINACEAE)

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Abstract

We conducted habitat, germination, and population genetic studies to inform management priorities for *Phacelia cookei* Constance & Heckard (Boraginaceae), a diminutive annual herb known from only four populations near Mt. Shasta in Siskiyou Co., California. Habitat surveys characterized soil, vegetation, and ground cover of extant populations and attempted to identify potentially suitable, but uncolonized, habitat. We were unable to distinguish any sites based on tests of soil characteristics. *Nama densum* Lemmon occurred at all sites where *P. cookei* was present. We identified several areas near existing populations that appeared to be suitable, but uncolonized, habitat. We tested the effects of various factorial combinations of after-ripening, scarification, stratification, and variable germination temperatures on breaking seed dormancy. Seed viability by tetrazolium tests ranged between 89% and 93%, but the highest germination from any treatment combination was 14% after adjusting for seed viability. We resolved 19 putative allozyme loci, two of which were polymorphic. Apparent genetic diversity was low both within and among the three sampled populations compared to similar endemic species, and populations were genetically similar. Management plans could consider attempting to expand existing populations by sowing seeds from existing populations into similar habitat.

Key Words: Conservation, endemism, genetic diversity, habitat, *Phacelia cookei*, rarity, seed dormancy.

Rare plants are an important component of biologically diverse ecosystems. Rare species can be widespread but infrequent throughout their distribution or have a very narrow geographical range with varying abundance; the latter are considered endemic species (Rabinowitz 1981). Narrow endemism often appears to be the result of adaptation to environments that are geographically restricted (Mason 1946). Endemic species with few individuals and/or populations are often especially susceptible to extinction due to their low numbers as well as their habitat specificity. Biodiversity hotspots, such as the California Floristic Province, where nearly 27% of the plant species are endemic, hold particularly high levels of endemism and have been targeted for focused conservation efforts (Myers et al. 2000).

Interactions between plants in a community, biotic factors, and abiotic stress can be important in determining community structure and species survival. Knowledge of these interactions is particularly important for understanding the distribution and protection of endemic plant species (Callaway and Walker 1997; Pugnaire and Luque 2001; Reynolds et al. 2003). A coarse-scale environmental component that can limit plant distribution is soil; edaphic endemism, for example, has been documented in several rare species (Kruckeberg 1954; Fiedler 1985; Cowling et al. 1994). Conservation programs, moreover, have used disturbance methods such as fire or soil disturbance to manage rare species (Preston and Whitehouse 1986; Hobbs and Huenneke 1992; Pendergrass et al. 1999). Intermediate levels of disturbance are thought to maintain the highest species diversity (Connell 1978), and diverse plant communities are more likely to support rare species (Myers et al. 2000). The response of rare species to disturbance should be carefully evaluated to avoid establishment of non-native species, which can out-compete or displace rare species, particularly in disturbed areas (Hobbs and Huenneke 1992; McIntyre and Lavorel 1994; Wilcove et al. 1998; Huston 2004).

Identifying appropriate germination cues can help to determine the feasibility of reintroduction of seeds from rare plants into uncolonized habitats in the field (Cochrane et al. 2002). Germination cues thus identified could also be used to predict germination time in the field based on local weather patterns. Germination rates tend to be

lower in small populations (Menges 1991; Keller and Waller 2002; Kochánková and Mandák 2009). For example, population size and germination rate were correlated in the recently fragmented prairie species *Silene regia* Sims (Menges 1991). Populations of this species with more than 150 individuals had consistent germination rates greater than 85%. Populations with fewer than 150 individuals had variable germination rates within and between populations, possibly due to recent fragmentation and/or inbreeding depression. Small endemic populations may have difficulty increasing population size or simply persisting due to decreased germination rates; so understanding their germination requirements could inform management and prevent extinction.

Understanding the genetic structure of a population can also inform management decisions to enhance genetic variability and survival (Ellstrand and Elam 1993; Alsos et al. 2002; Dolan et al. 2004). Population genetic studies can determine the degree to which populations are genetically distinct and can have important implications for managing narrow species. For example, the introduction of seed without careful consideration of population genetic structure and fitness can result in harmful changes to populations (Millar and Libby 1989). Experimental introduction of plants from multiple seed stocks could increase the success of re-establishment via heterosis and increased genetic diversity (Barrett and Kohn 1991); however, such practices risk disrupting locally adapted gene combinations (Antonovics 1976; Simberloff 1988; Barrett and Kohn 1991). Re-establishing populations from mixed seed sources will decrease population differentiation, but successfully re-established populations with long-term survival will likely differentiate again over time (Barrett and Kohn 1991). Introducing seeds from ecologically similar, but genetically diverse populations to maintain environmental adaptations and increase genetic diversity within the populations is recommended (Godt et al. 1996). Conservation plans that are approached experimentally increase our knowledge of population biology and can inform specific goals of future conservation plans (Barrett and Kohn 1991).

We studied *Phacelia cookei* Constance & Heckard (Boraginaceae), a rare annual limited to a 5 km^2 area near Mt. Shasta in Siskiyou County, California. The purpose of this study was to determine the habitat preferences, germination requirements, and population genetic structure to improve species management plans.

Methods

Species Description

Phacelia cookei is an annual herb, 2–15 cm tall, which occurs in areas of high disturbance in and along dirt roads where there is low surface organic matter and low competition from other plants (Horner-Till 1982). Flowers are 1–1.5 mm wide and can produce seeds in a growth chamber free of pollinators (Patterson unpublished), indicating that *P. cookei* is likely capable of self-fertilization. The species completes its life cycle between late May and early August. Populations occur on 0–35% slopes, and at elevations of 1330–1650 m in Siskiyou Co., California. The community associations in which it has been found include the *Chrysothamnus nauseosus* association, *Pinus ponderosa* association, and *Arctostaphylos patula*/*Ceanothus velutinus* association (Sawyer and Keeler-Wolf 1995; Barbour et al. 2007). Species nomenclature herein follows Baldwin et al. (2012).

A previous study examined the habitat characteristics and seed dispersal of *P. cookei* (Horner-Till 1982). The only populations found were in highly disturbed areas, primarily annually graded road banks, unused road beds, fuel breaks, or other frequently disturbed sites, despite intensive surveys of the area. Approximately 50% of seeds produced were found to fall beneath the parent plant, with the remainder held within the plant and dispersing with senesced plant tissue by wind. Mark-recapture studies revealed that whole plants and plant fragments can function as wind-tumbled diaspores, with some fragments moving as much as 22 m within a day. A series of pilot seed-germination experiments was also conducted, in which light duration, light intensity, temperature, scarification, and stratification were manipulated to identify germination cues. Treatment combinations were not applied consistently, however, and all treatments had germination $<11\%$, with most treatments ranging from 0–2.5% (Horner-Till 1982).

Since the Horner-Till (1982) study, the number of known populations of *P. cookei* (40 reported) has decreased. These populations were not mapped, but a few (including the experimental populations used in this study) were documented in herbaria and likely include populations that Horner-Till studied. During surveys conducted on May 25 and 26, 2008, 10 populations were located (Edwards and Schierenbeck unpublished), including several that were not previously documented in herbaria. Habitat descriptions in Horner-Till (1982) indicate the occurrence of periodic fires, and firebreaks in the area contained populations of *P. cookei*.

Four spatially distinct populations were identified for this study, all of which were within a short distance and ranged in elevation from 1107 m–1408 m. Populations 1 and 4 were separated by approximately 2.4 km, and populations 6 and 7, which were closely adjacent, were approximately 5.3 km from population 1 (Table 1).

TABLE 1. SITE LOCATIONS AND SITE CATEGORIZATION FOR A SURVEY OF *PHACELIA COOKEI* HABITAT IN SISKIYOU CO., CALIFORNIA, USA, AUGUST 2009. *Indicates *Phacelia cookei* was present at the survey site.

Site	Location (UTME, UTMN)	Site category
01*	0566220, 4600778	historical occurrence
02	0566895, 4599400	historical occurrence
03	0566956, 4599347	historical occurrence
04*	0567069, 4598387	historical occurrence
05	0567639, 4597927	potential habitat
06*	0562769, 4598057	historical occurrence
07*	0562978, 4597937	historical occurrence
08	0563037, 4597904	potential habitat
09	0561107, 4599508	potential habitat
10	0560233, 4599200	volcanic rock pit
11	0559970, 4598891	volcanic rock pit
12	0562392, 4597949	potential habitat
13	0562466, 4597915	potential habitat
14	0562629, 4598024	potential habitat
15	0562743, 4589015	potential habitat

Habitat Survey

Habitat was surveyed for associated vegetation; percent cover of bare ground, litter, and vegetation; and soil characteristics. Fifteen sites were surveyed on August 17 and 18, 2009, following the California Native Plant Society (CNPS) Vegetation Rapid Assessment Protocol (CNPS Vegetation Committee 2007), for associated species, elevation, and habitat and vegetation descriptions. At each site, the 10–20 most common or abundant species were recorded within each stand; stands were defined by their structural and compositional integrity (see CNPS Vegetation Committee 2007 for more information). In addition to rapid assessment surveys, percent cover of bare ground, litter, and vegetation was measured within three to five 0.5 m^2 quadrats haphazardly placed in areas considered to be suitable habitat for *P. cookei*. A 0.5 m^2 quadrat was used because habitat areas were typically small or along narrow road margins. Vegetation cover was recorded as the percent cover of individual species within 0.5 m^2. Habitat data (percent bare ground, litter, and vegetation) were significantly heteroscedastic among site categories (see below), even after transformation, thus data were compared among site categories using a non-parametric Kruskal-Wallis test.

Surveyed sites included: (a) locations in which *P. cookei* currently or historically occurred ("historical locations") based on herbarium collection records and recent surveys (conducted by Edwards and Schierenbeck, unpublished); (b) areas of potentially suitable habitat ("potential habitat"); and (c) pits with rock of volcanic origin that had been mined for road construction ("volcanic rock pits"). All sites were within an area of approximately 5 km^2 (Table 1). Potential habitat locations were chosen based on apparent similarity to historical sites. Potential habitat was considered similar to historical sites if it was within the geographical range of *P. cookei* and had two or more of the following characteristics: a high level of disturbance as evidenced by tire tracks, a low percent canopy cover, low amounts of competing vegetation and/or litter, and/or a sandy substrate. Volcanic rock pits were surveyed because extant *P. cookei* populations are closely associated with dirt road margins that are maintained with a layer of volcanic rock. Seed could have been distributed in the volcanic roadbed material or it could be otherwise critical to habitat soil properties. The volcanic rock pits are located near the *P. cookei* sites and produce rocks very similar to that found in the roadbed.

A soil sample was collected from the approximate center of each survey site for analysis to determine whether soil texture and nutrient content limit colonization into new habitat. Soil was air-dried and passed through a 2 mm sieve to remove gravels. Cations were extracted using pH 7.0 ammonium acetate (Thomas 1982), with quantification of Ca, Mg, Na, and K by atomic adsorption/emission spectroscopy and data converted to meq/100 g. Extractable cations are a robust measurement of potentially available forms (Thomas 1982). The soil cation exchange capacity (CEC) was gauged by the sum of extractable cations. Immiscible displacement (ID) was used as a proxy for cations and anions in the soil-solution (Mubarak and Olsen 1976) with cations Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} quantified by atomic adsorption/emission spectroscopy and anions Cl^{-} and SO_4^{-2}, and ortho-P by ion chromatography. Cations and anions in the soil solution are readily available for plant root uptake. Given the pH of these soils, we employed the Bray method to gauge phosphorus availability with quantification by vanomolybdate chemistry, a colorimetric reagent, using flow injection to use small samples (Bray and Kurtz 1945). The ratio of Bray extractable to ID phosphorus was calculated to more accurately measure the amount of phosphorus in the soil.

Nitrogen availability was quantified by KCl-extraction (Bundy and Meisinger 1994), with quantification of ammonium and nitrate using the Lachat autoanalyzer. The mole percent of ammonium in KCl extracts was determined as it often is related by plant succession and disturbance history (R. Blank, USDA Agriculture Research Service, Reno, NV, personal communication). Soil pH was measured twice: in a 0.01 $CaCl_2$ matrix and in a NaF matrix. The reaction of F- with poorly ordered hydrous oxides causes an increase in pH relative to that measure in aqueous matrix and can be a good proxy for levels of volcanic ash in the sample (R. Blank,

personal communication). To assess soil texture, coarse weight, fine weight, total weight, and percent coarse fragments were measured. Percentage water at saturation was measured to determine the water holding capacity of the soil. Soil tests were conducted at USDA Agriculture Research Service in Reno, Nevada.

Soil test data did not conform to assumptions of parametric tests, thus we compared soil across site categories using non-parametric Kruskal-Wallis tests.

Seed Germination

To clarify and possibly improve upon the methods reported by Horner-Till (1982), we conducted two experiments to identify cues to break seed dormancy, information that could then be used to estimate germination windows in the field and determine the feasibility of *ex situ* propagation.

Collection and seed separation. Twenty-five dried plants were collected on August 9, 2008 from populations 1 and 4, and an additional 25 from populations 6 and 7 combined, as the two sites were in close proximity and sparsely populated. Seeds were separated from dried plant material and stored in coin envelopes labeled by parent plant at room temperature for three months.

Seed viability. Four samples of 50 seeds from each population were tested for viability using 1% 2,3,5-triphenyl tetrazolium (Lakon 1949). Seeds were pierced and soaked in a 1% tetrazolium solution overnight in the dark. Seeds were considered viable if embryos stained dark pink. Germination rates from dormancy experiments were divided by mean viability within each population to correct for proportion viable.

Germination experiment 1. Seeds from three different populations were subjected to 14 different treatment combinations in a factorial design including acid scarification (5, 10, or 15 min), cold-moist stratification (present or absent), and temperature during germination (cycling 5°C in the dark for 12 hours followed by 25°C or 30°C in the light; Table 2). One seed from each maternal plant was used for each treatment combination (approximately 20 per population). Seeds were soaked in concentrated sulfuric acid for 5, 10, or 15 minutes and then rinsed for two minutes in deionized water. Treated seeds were allowed to dry overnight. Seeds assigned to receive cold-moist stratification were placed in beakers with moist perlite on October 31, 2008, and stored at 4°C for five weeks. Seeds that did not receive cold-moist stratification were treated on December 12, 2008.

Following scarification or stratification, seeds from within a population and treatment combination were placed on moist filter paper in 9-cm diameter Petri dishes. Each treatment combination, therefore, included three Petri dishes, one from each population, each of which contained a single seed from each maternal family collected. Petri dishes were incubated for four weeks at one of two fluctuating temperature regimes. In one group, seeds were held for 12 hours each day at 5°C and then temperature was increased to 25°C for the reminder of each 24-hour period. The second group was held 12 hours each day at 5°C and then the temperature was increased to 30°C for the remainder of the 24-hour period.

TABLE 2. TREATMENTS APPLIED TO *PHACELIA COOKEI* SEEDS IN TESTS TO BREAK DORMANCY ("GERMINATION EXPERIMENT 1"—SEE METHODS). Treatments included sulfuric acid scarification, cold-moist stratification, and temperature fluctuations, in which 24-hr cycles consisted of 12 hr at the low temperature and 12 hr at the high temperature indicated.

Acid scarification	Cold moist stratification	Temperature
5 min	5 wk	5°C/25°C
10 min	5 wk	5°C/25°C
15 min	5 wk	5°C/25°C
0 min	none	5°C/25°C
5 min	none	5°C/25°C
10 min	none	5°C/25°C
15 min	none	5°C/25°C
5 min	5 wk	5°C/30°C
10 min	5 wk	5°C/30°C
15 min	5 wk	5°C/30°C
0 min	none	5°C/30°C
5 min	none	5°C/30°C
10 min	none	5°C/30°C
15 min	none	5°C/30°C

Germination experiment 2. Germination success was relatively low in the first experiment, so we also conducted a second germination experiment with an expanded range of treatments in an attempt to identify germination cues. During the second experiment, we tested the effects of two after-ripening treatments in conjunction with cold-moist stratification on germination. Twelve different treatment combinations were applied to the seeds (Table 3). Treatments were chosen using the Horner-Till thesis (1982) and a germination decision tree (Meyer 2006). Seeds were placed on 12 separate 50-mm diameter Petri dishes per individual plant. Population 1 had 16 individuals, population 4 had 14 individuals, and Populations 6 and 7 had 25 individuals each included in this experiment, for a total of 660 plates. The number of seeds in each dish varied between 1–10 seeds depending on the number available per plant. All seeds from a single plant assigned to receive the 40°C treatment were combined into a single sealed vial to prevent loss of seed moisture, after which they were separated into Petri plates for cold stratification.

TABLE 3. TREATMENTS APPLIED TO *PHACELIA COOKEI* SEEDS IN TESTS TO BREAK DORMANCY ("GERMINATION EXPERIMENT 2"—SEE METHODS). Treatments included after-ripening, cold stratification, and temperature fluctuations, in which each 24-hr cycle consisted of 12 hr at the low temperature alternating with 12 hr at the high temperature indicated.

After-ripening (2 weeks)		Cold stratification	
Temp.	Moisture	at 2°C	Temperature
----	----	8 wk	2°C/10°C
----	----	8 wk	5°C/25°C
----	----	12 wk	2°C/10°C
----	----	12 wk	5°C/25°C
15°C	wet	8 wk	2°C/10°C
15°C	wet	8 wk	5°C/25°C
15°C	wet	12 wk	2°C/10°C
15°C	wet	12 wk	5°C/25°C
40°C	dry	8 wk	2°C/10°C
40°C	dry	8 wk	5°C/25°C
40°C	dry	12 wk	2°C/10°C
40°C	dry	12 wk	5°C/25°C

For both experiments, the criterion for germination was the protrusion of the radicle, which was visible to the naked eye. The percentage germination within each Petri dish was compared among populations and treatments using Kruskal-Wallis tests, as data did not conform to assumptions for parametric tests.

Isozyme Variation

A survey of allozyme variation was conducted to characterize population genetic variation within and among populations.

Sample collection and extraction. Leaves from 50 living plants were collected from each of populations 1 and 4, and 24 samples were collected from populations 6 and 7 (sites 6 and 7 were combined into one population due to the low number of individuals in each population and the close physical proximity of the two populations) on June 8, 2009 and stored in a plastic bag on ice or refrigerated up to 48 hours until extraction. All tissue samples were crushed in a chilled ceramic spot plate using a glass pestle with an extraction buffer modified from Broyles and Wyatt (1990). The extract was filtered through Miracloth, adsorbed onto 3 × 10 mm wicks cut from Whatmann 3MM chromatography paper, and stored at −70°C until electrophoresis was performed. Wicks were loaded onto 12.5% hydrolyzed potato starch gels and subjected to horizontal electrophoresis following Soltis and Soltis (1989).

Gel systems. The following isozymes and buffer combinations were used to estimate genetic variability within and between populations. A tris-citrate buffer (pH8.0) (Meizel and Markert 1967) was used to resolve isocitrate dehydrogenase (IDH:EC:1.1.1.41), 6-phosphoguconate (PGD:EC:1.1.1.44), glyceraldehyde-6-phosphate dehydrogenase (G6PDH:EC:1.1.1.49), glutamate dehydrogenase (GDH:EC:1.4.1.2), and glyceraldehyde-3-phosphate dehydrogenase (G-3PDH: EC:1.2.1.9). A histidine-citrate buffer (pH 7.0) (Fildes and Harris 1966) was used to resolve phosphoglucomutase (PGM:EC:5.4.2.2), menadione reductase (MNR:EC:1.6.99), malic enzyme (ME:EC:1.1.1.40), phosphglucoisomerase (PGI: EC:5.3.1.9), and triose-phosphate isomerase (TPI: EC:5.3.1.1). A tris-borate-EDTA buffer (pH 8.6) (Markert and Faulhaber 1965) was used to resolve diaphorase (DIA:EC:1.6.2.2), UTP-glucose 1-phosphate uridylyltranferase (UGPP: EC:2.7.7.9), aldolase (ALD:EC:4.1.2.13), glutimate oxaloacetate transaminase (GOT:EC:2.6.1. 1), and shikimate dehydrogenase (SHK:EC:1.1.1. 25). All enzyme assays followed Wendel and Weeden (1989) except UGPP, which followed Manchenko (1994). Stains were incubated at 37°C in the dark until bands appeared.

Analysis. POPGENE (Yeh and Boyle 1997) was used to calculate allele frequencies, F-statistics, geneflow, heterozygosity, and the effective number of alleles. F-statistics were calculated following Weir (1990) to determine deviations from Hardy-Weinberg equilibrium. Total gene diversity (H_t) and mean diversity within populations (H_s) were calculated following Nei (1973, 1978). The effective number of alleles was calculated following Hartl and Clark (1989).

RESULTS

Habitat Survey

Percentage bare-ground cover did not differ among types of habitat (mean [SD] historical occurrence = 74.59 [34.33], potential habitat = 77.86 [31.79], and volcanic rock pits = 85.83 [22.45]; $H = 2.89$, $P = 0.2$). Percentage cover by litter also did not differ among sites (mean [SD] historical occurrence = 20.18 [34.24], potential habitat = 17.38 [23.58], and volcanic rock pits = 2.50 [4.18]; $H = 3.71$, $P = 0.2$). Percentage vegetation cover, however, was significantly higher in the historical occurrence and volcanic rock pit sites than in the potential habitat (mean [SD] historical occurrence = 14.64 [28.37], volcanic rock pits = 11.67 [19.15], potential habitat = 4.05 [11.53]; $H = 8.28$, $P = 0.016$).

Nama densum Lemmon was the species most closely associated with *P. cookei* as it was found at all sites that had *P. cookei* present and was not found at any site without *P. cookei* (Table 4), and was thus the species most closely associated with *P. cookei.* Other species commonly associated with *P. cookei* were *Bromus tectorum* L., *Gayophytum heterozygum* F.H. Lewis & Szweyk.,

TABLE 4. PERCENT COVER OF PLANTS PRESENT AT SITES SURVEYED IN A STUDY CHARACTERIZING HABITAT OF *PHACELIA COOKEI*. Site numbers correspond to locations in Table 1. Missing values indicate that the species was not present at that location. Surveys were conducted in Siskiyou County, CA, USA in August 2009.

Taxon	Site number														
	1	4	6	7	2	3	5	8	9	12	13	14	15	10	11
Achillea millefolium									1						
Agrostis idahoensis	1				1	1				2			1		1
Arctostaphylos nevadensis							1								
Artemisia tridentata	5				5			5	3	20	5		1		1
Bromus carinatus												1			
Bromus tectorum	1	1	1		1		1			3	30	1	1		
Castilleja arachnoidea											1				
Ceanothus velutinus							1								
Chaenactis douglasii							1								
Chamaesyce glyptosperma		1													
Chenopodium atrovirens	1		1					1							
Collomia grandiflora					1	1									
Cryptantha intermedia													1		
Delphinium nuttallianum							1								
Dysphania botrys		5													
Elymus elymoides	1				1	1	1								
Epilobium sp.	1				1		1								
Ericameria nauseosa	5	5			1						3		1	1	
Erigeron filifolius	1	1	1		1		1	1							
Eriogonum baileyi			1												
Eriogonum nudum		1													
Eriogonum umbellatum				1											
Eriophyllum lanatum var. *obovatum*															1
Gayophytum heterozygum	1	1		1		1	1							1	
Gilia sinuata					1						1				
Gutierrezia microcephala	1	1	5				1								
Juniperus occidentalis															1
Linanthus pungens	1				1				2						
Mentzelia albicaulis		1	1												
Nama densum	1	1	5	1											
Nicotiana attenuata							1								
Penstemon laetus var. *sagittatus*		1													
Phacelia cookei	2	1	<1	<1											
Phacelia hastata		5					1								
Pinus ponderosa	1					75									
Pterospora andromedea						1									
Purshia tridentata	15				5	5		5							
Ribes cereum						5									

TABLE 4. CONTINUED.

Taxon	Site number													
	1	4	6	7	2	3	5	8	9	12	13	14	15	10	11
Sisymbrium altissimum			1												
Stephanomeria sp.		1													
Stipa occidentalis	1				1	1								1	
Verbascum thapsus														3	
Total vegetative cover	40	26	17	4	20	91	12	12	6	25	40	2	5		4

Erigeron filifolius Nutt., and *Gutierrezia microcephala* (DC.) A. Gray, which were found at three of the four sites that had *P. cookei* present. *Stipa occidentale* S. Watson, *Agrostis idahoensis* Nash, *Artemisia tridentata* Nutt., *Chenopodium atrovirens* Rydb., *Dysphania botrys* (L.) Mosyakin & Clemants, *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom & G.I. Baird, *Elymus elymoides* (Raf.) Swezey, *Epilobium* sp., *Linanthus pungens* (Torr.) J.M. Porter & L.A. Johnson, *Penstemon laetus* var. *sagittatus* (D.D. Keck) McMinn, *Phacelia hastata* subsp. *compacta* (Brand) Heckard, *Pinus ponderosa* Douglas ex Lawson & C. Lawson, *Purshia tridentata* (Pursh) DC., and *Sisymbrium altissimum* L. were present at half of the sites that had *P. cookei* present.

Bromus tectorum was documented at nine of the 14 sites, three of which also had *P. cookei*. Most sites, including those with *P. cookei*, had very low percent cover (1%) of *Bromus tectorum,* except site 13 where it reached 30%. Disturbance, as evident from tire tracks and crushed vegetation, was observed at all sites with *P. cookei* except site 4, which was near railroad tracks.

In general, soils at the study sites were sandy in texture, which is characteristic of Delany and Oosen-Avis soil families in the area (USDA/NRCS 2010). The mean coarse weight at the volcanic rock pits, however, was higher than the course weight of potential habitat and historic habitat (Table 5), likely because the volcanic rock pits are sources of a rocky roadbed material whereas the historic and potential habitat areas are relatively sandier. In addition, mean pH was lower at volcanic rock pit sites than at potential habitat and historical occurrence sites (Table 5). Nonetheless, no significant differences were detected among sites surveyed for any of the soil characteristics we measured (Table 5). Historical sites without extant *P. cookei* populations did not differ significantly from those with *P. cookei* present, so they were grouped for analysis. The similarities among sites suggests that soil characteristics do not limit the distribution of *P. cookei*, at least within its known geographical range.

Seed Germination

Viability testing. A high percentage of seeds stained positive for enzyme activity following the tetrazolium test (positive test observed in 89%, 90%, and 93% of seeds respectively, from populations 1, 4, and 6/7).

Germination experiment 1. We found no significant differences in percentage germination among any of the population by treatment combinations ($H = 0.07$, $P = 0.9$), so we combined data across populations for subsequent analyses to increase our power to detect treatment effects. Nonetheless we found no difference

TABLE 5. MEAN (SD) RESPONSES FROM SOIL ANALYSES CONDUCTED DURING A SURVEY OF HABITAT OF *PHACELIA COOKEI*. Sites were categorized as historical occurrence (n = 6), potential habitat (n = 7), or volcanic rock pit sites (n = 2; see Table 1). Soil samples were collected during August 2009 in Siskiyou Co., California. See Methods for explanation of soil analysis. Test statistic was calculated using the non-parametric Kruskal-Wallis test to determine significant differences in ranked means. † $0.1 > P > 0.6$.

Soil analysis	Historical occurrence. Mean (SD)	Potential habitat. Mean (SD)	Volcanic rock pits. Mean (SD)	Test stat. (H)
meq/100 g acetate Ca	2.15 (2.22)	1.09 (0.60)	3.40 (3.71)	0.59
meq/100 g acetate Mg	0.57 (0.72)	0.16 (0.08)	0.98 (0.91)	3.21
meq/100 g acetate K	0.29 (0.23)	0.09 (0.05)	0.34 (0.34)	3.37
meq/100 g acetate Na	4.51 (0.75)	4.62 (0.21)	4.65 (0.59)	0.0
CEC by sum of cations (meq/100 g)	7.53 (2.48)	5.96 (0.64)	9.36 (4.36)	4.34
ug/g Bray P as P	79.48 (57.4)	93.69 (34.03)	17.47 (18.99)	4.03
ug/mL ID Ca	3.13 (1.64)	7.54 (7.48)	3.75 (3.75)	1.14
ug/mL ID Mg	1.63 (1.65)	2.14 (1.62)	2.65 (2.33)	0.71
ug/mL ID K	6.90 (5.40)	5.03 (2.62)	5.85 (6.72)	0.35
ug/mL ID Na	2.42 (1.87)	2.27 (1.22)	2.60 (0.42)	0.7
coarse wt.	22.24 (26.28)	34.37 (27.92)	221.76 (164.07)	5.67†
fine wt.	285.38 (106.46)	393.66 (88.28)	310.8 (64.45)	3.84
total wt.	307.62 (123.90)	428.03 (75.15)	532.52 (228.52)	3.75
% coarse frag	6.47 (6.00)	8.52 (7.45)	38.58 (14.26)	5.16†
ppm KCl NH4	0.61 (0.23)	1.10 (1.19)	1.07 (1.00)	0.68
ug/g KCL (mmol/kg)	1.81 (0.68)	3.25 (3.56)	3.19 (3.00)	0.49
KCl NH4 (mmol/kg)	0.10 (0.04)	0.18 (0.2)	0.18 (0.17)	0.49
ppm KCl NO3	2.24 (1.93)	4.33 (3.63)	3.7 (3.86)	1.54
ug/g KCl NO3	6.58 (5.66)	12.70 (10.63)	11.08 (11.61)	0.54
KCl NO3 (mmol/kg)	0.11 (0.09)	0.20 (0.17)	0.18 (0.19)	1.53
total KCl N (mmol/kg)	0.21 (0.12)	0.39 (0.3)	0.36 (0.35)	0.99
mole %KCl as NH4	57.75 (24.64)	50.36 (27.04)	52.39 (5.26)	1.54
ug/ml ID Cl	1.97 (1.12)	1.28 (0.79)	1.49 (1.07)	1.87
ug/mL ID SO4	2.34 (1.39)	2.53 (2.32)	4.25 (3.90)	0.75
ug/ml ID P as P	0.29 (0.4)	0.46 (0.41)	0.26 (0.18)	2.06
CaCl pH	5.79 (0.2)	5.50 (0.45)	6.26 (0.43)	4.23
NaF pH	9.07 (0.47)	8.52 (0.62)	8.51 (0.41)	3.56
pH diff	3.28 (0.5)	3.02 (0.32)	2.26 (0.02)	5.63†
% water at saturation	28.85 (6.97)	25.84 (2.7)	26.41 (6.25)	0.22
ug/g ID P as P	0.10 (0.15)	0.12 (0.11)	0.07 (0.06)	0.91
ratio Bray to ID P	2085.07 (1518.57)	2267.67 (3725.59)	199.06 (81.95)	3.73

in percentage germination between the two germination temperature cycles (5/25°C vs. 5/30°C) ($H = 0.20$, $P = 0.66$; Table 6) or among scarification times in acid ($H = 2.39$, $P = 0.50$; Table 6) (we note, however, that the 15-min treatment severely damaged the seeds). We did not find percentage germination in the acid-only versus the acid-and-cold-stratification treatments to differ significantly ($H = 0.15$, $P = 0.70$; Table 6). Likewise, we found no significant differences among treatments when comparing all ten treatment combinations ($H = 11.40$, $P = 0.6$).

Germination experiment 2. Percentage germination did not differ among any of the population by treatment combinations ($H = 0.17$ to 2.51, $P = 0.3$ to 0.9; Table 7), so data were combined across populations for subsequent analyses to increase power to detect treatment effects.

TABLE 6. MEAN (SD) PERCENT GERMINATION OF *PHACELIA COOKEI* SEEDS IN TESTS OF DORMANCY CUES ("GERMINATION EXPERIMENT 1"—SEE METHODS). Germination values were corrected for mean population seed viability, as estimated by tetrazolium tests.

Treatment	Percent germination. Mean (SD)	Test statistic (H)
Acid scarification time		2.39
0 min	2.07 (2.31)	
5 min	6.05 (12.02)	
10 min	1.09 (2.03)	
15 min	0.88 (3.03)	
Cold stratification		0.15
none	3.2 (8.47)	
5 wk	1.8 (4.21)	
Germination temperature		0.20
5/25°C	3.34 (9.06)	
5/30°C	1.83 (3.91)	

TABLE 7. MEAN (SD) PERCENT GERMINATION OF *PHACELIA COOKEI* SEEDS IN A TEST OF DORMANCY CUES ("GERMINATION EXPERIMENT 2"—SEE METHODS). Treatments included after-ripening, cold stratification, and germination temperature cycle. * $P < 0.05$.

Treatment	Percent germination. Mean (SD)	Test statistic (H)
After-ripening		1.42
none	3.40 (4.09)	
warm/moist (15°C)	14.40 (14.83)	
hot/dry (40°C)	3.28 (4.97)	
Cold stratification (2°C)		0.03
8 wk	8.40 (12.25)	
12 wk	5.68 (8.24)	
Germination temp.		7.41*
5/25°C	13.35 (11.10)	
2/10°C	0.70 (1.71)	

Percentage germination was not affected by after-ripening treatment ($H = 1.42$, $P = 0.50$; Table 7) or length of cold stratification ($H = 0.03$, $P = 0.87$; Table 7). Percentage germination was higher among seeds that were exposed to a 5/25°C temperature cycle than those in the 2/10°C temperature cycle ($H = 7.41$, $P = 0.01$; Table 7).

No germination was observed under the following conditions: (1) 8 weeks cold stratification/germinated at 2/10; (2) 12 weeks of cold stratification/germinated at 2/10°C; (3) warm after-ripening/8 weeks cold stratification/germinated at 2/10°C; (4) hot after-ripening/8 weeks cold stratification/germinated at 2/10°C; and (5) hot after-ripening/12 weeks cold stratification/germinated at 2/10°C.

Isozyme Variation

Seventeen of the 19 putative allozyme loci resolved were monomorphic (Table 8). Only DIA and MNR were polymorphic. Both DIA and MNR stain for a varied group of flavoproteins with little specificity and in some plants these two markers stain for the same allozyme, or there may be overlap between the stains (Soltis and Soltis 1989). However, banding patterns were dissimilar for *P. cookei*, thus they were treated as distinct loci. All populations had 10.53% polymorphic loci. The mean number of alleles at each population was 1.16. The effective number of alleles for populations 1, 4, and 6/7 was 1.06, 1.13, and 1.14 respectively. The effective number of alleles did not deviate significantly from the mean number of alleles. Allele frequencies did not significantly deviate from Hardy-Weinberg equilibrium (DIA: $\chi^2 = 1.99$, df = 1, $P = 0.2$; MNR: $\chi^2 = 7.24$, df = 3, $P = 0.06$).

TABLE 8. ALLELE FREQUENCIES AT TWO VARIABLE ISOZYME LOCI IN THREE POPULATIONS OF *PHACELIA COOKEI*. Sample size per locus: Pop 1 (50); Pop 4 (50); Pop 6/7 (24). Mean expected heterozygosity (H_s) and mean number of alleles per locus per polymorphic loci (AP) are shown for each population.

Locus	Allele	Populations 1	4	6/7
DIA	a	0.18	0.39	0.44
	b	0.82	0.61	0.56
MNR	a	0.08	0.25	0.23
	b	0.20	0.24	0.27
	c	0.72	0.51	0.5
H_s		0.04(0.12)	0.06(0.18)	0.06(0.18)
AP		2.5	2.5	2.5

TABLE 9. GENETIC DIVERSITY, AS ESTIMATED BY ALLOZYME VARIATION, IN THREE *PHACELIA COOKEI* POPULATIONS. Jackknife estimates of heterozygote deficit within individuals (F_{is}), observed total (H_t) and population-level (H_s) heterozygosities were calculated according to Nei (1973) using GenePop. Estimates for two polymorphic allozyme loci, as well as population mean estimates, are provided.

Locus	F_{is}	H_T	H_s
Population 1			
MNR	−0.265	0.560	0.435
DIA	0.007	0.240	0.295
Pop. means	−0.095	0.042(0.14)	0.038(0.12)
Population 4			
MNR	−0.194	0.740	0.620
DIA	0.117	0.420	0.476
Pop. means	−0.059	0.061(0.19)	0.058(0.17)
Population 6/7			
MNR	−0.335	0.833	0.624
DIA	−0.101	0.542	0.492
Pop. means	0.187	0.072(0.22)	0.057(0.18)
All populations			
MNR	−0.271	0.686	0.564
DIA	0.049	0.371	0.431
means	−0.134	0.056(0.18)	0.052(0.16)

Individual-locus estimates of inbreeding (F_{IS}) were negative for MNR, as well as for the mean for all populations (Table 9). F_{IS} estimates for DIA in population 1 and 4 were positive but negative in population 6/7. In contrast, F_{IS} estimates for MNR were consistently negative. Jackknife estimates of genetic differences among populations (F_{ST}) for MNR and DIA were 0.0292, and 0.0561 respectively, and mean F_{ST} was 0.0409. Due to low allozyme variation observed, these estimates should be considered provisional.

DISCUSSION

We identified a number of areas near existing populations that appeared to be suitable (low vegetation and litter cover and high bare ground), but uncolonized, habitat. We have identified treatment combinations that can break seed

dormancy, albeit at low frequencies (<15%). Thus, managers could consider attempting to expand existing populations into some of these new areas by sowing seeds in to areas with low vegetation and litter cover. Genetic variation appears to be limited in *P. cookei*, which may ultimately limit its prospects for recovery.

Habitat

Associated species and percent cover of bare ground, litter, and vegetation may be useful for locating potential habitat and new populations. Bourg et al. (2005), for example, successfully used classification and regression tree (CART) modeling and geographic information systems (GIS) computer software with habitat characteristics, such as forest type and elevation, to locate eight new occupied habitat patches of the rare forest species *Xerophyllum asphodeloides*. We found *Nama densum* to be most frequently associated with *P. cookei*, suggesting that targeting habitat with *N. densum* may also help protect *P. cookei*.

Soil did not differ among the categories of sites we examined, which suggests that soil characteristics might not be limiting the spread of *P. cookei* within its geographical range. The volcanic rock roadbed material, moreover, was not significantly different than the sand substrate, so a sandy substrate may not be a habitat requirement. The current limited distribution of *P. cookei* may instead reflect the limitations of its gravity and tumbleweed-type seed dispersal (Horner-Till 1982), or perhaps the distribution of the Delaney and Oosen-Avis soil families (USDA/NRCS 2010). Another characteristic that might limit the expansion of *P. cookei* populations is the extent of bare soil or disturbance in areas adjacent to extant populations. Most extant populations had signs of disturbance and all had extensive bare ground and low percent cover of vegetation and litter. We did not measure the amount of bare soil or disturbance in areas other than the surveyed sites, but qualitatively these characteristics appeared to be distinctive in *P. cookei* habitat.

Germination

In a series of experiments, Horner-Till (1982) reported low germination success (all treatment combinations ≤11%). Similarly, germination was low in our experiments (<15%) and not strongly affected by physical (scarification) or physiological (stratification and after-ripening) treatments, suggesting *P. cookei* may have multiple dormancy cues that have yet to be identified or non-cue responsive dormancy (Meyer 2006). Seeds with non-cue responsive dormancy can be challenging to propagate from seed, so in situ, conservation measures are more likely to be successful. The 5/25°C temperature cycle, however, produced significantly higher germination rates than the 2/10°C temperature cycle. These temperatures are similar to the field temperatures reported by Horner-Till (1982) and may provide guidance for expanding the range into uncolonized, but apparently suitable, habitat.

Table 10. The Relationship of Geographical Range to Allozyme Variability in Plants (after Hamrick 1983).

Geographical region	No. of studies	H_t	H_s
Endemic	10	0.275	0.208
Narrow	31	0.261	0.177
Regional	38	0.238	0.154
Widespread	43	0.380	0.293
Phacelia cookei	1	0.056	0.053

Isozyme Variation

Overall, isozyme variation was very low, which suggests *P. cookei* harbors limited population genetic diversity. Hamrick and Godt (1989) found that endemic, short-lived, selfing dicots with gravity-dispersed seed (all characteristics of *P. cookei*) typically have low amounts of genetic diversity. The populations were very similar genetically (low F_{ST}), so there appears to be little concern for disrupting locally adapted genotypes if land managers dispersed seeds among populations or used them to colonize new sites. Population sizes could be expected to vary substantially from year to year, so if seeds are collected for this purpose, we suggest that the number of seeds harvested from each population be proportionate to the population size.

Our estimates of population genetic variation may have been low in part because samples of *P. cookei* tissue were collected during only one field season. Plants with small populations can maintain genetic diversity through a genetically diverse seed bank, so plants growing during one season need not be representative of total genetic diversity, including both living plants and dormant seeds (Del Castillo 1994). However, our sample sizes from populations 1 and 4 (50 individuals each) were adequate to sample at least some rare alleles across the 19 loci resolved, and the populations in that year contained several hundred individuals. Seventeen of those loci appeared to be fixed. Therefore, sampling across multiple years (Ellstrand and Elam 1993; Cabin et al. 1998; McCue and Holtsford 1998) may be unlikely to reveal much additional population variation. Nonetheless, since sampling across years could increase the probability of finding rare alleles, new populations established in suitable habitat should be seeded across

multiple years to maximize the evolutionary potential of each population established.

Hamrick (1983) surveyed the relationship between geographical range and allozyme variability (Table 10). As the geographic range increases, total allelic diversity, mean diversity within populations, and population differentiation increases (Table 10). H_t and H_s for *P. cookei* were significantly lower than what has been reported for endemic plants (Hamrick 1983). Very low diversity at the population level indicates decreased potential for evolutionary change in response to environmental change, which could pose a challenge for population persistence in the future.

The closest known relative to *P. cookei* is *P. keckii* Munz & I.M. Johnst. (Walden 2010). *Phacelia keckii* is an annual endemic to the Santa Ana Mountains that grows on volcanic soils in chaparral and knobcone pine communities (Stephenson and Calcarone 1999). After fire has occurred, *P. keckii* populations have been documented to increase in size (Stephenson and Calcarone 1999). Horner-Till (1982) documented the fire history of the area and speculated about the relationship between fire and *P. cookei*. Investigations into the role of fire and other sources of disturbance in *P. cookei* habitat may provide additional insights into management treatments beneficial to population growth.

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